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L1: Entry 67 of 78

File: USPT

Jun 10, 2003

US-PAT-NO: 6576455

DOCUMENT-IDENTIFIER: US 6576455 B1

TITLE: Structure-based designed herbicide resistant products

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kakefuda; Genichi	Yardley	PA		
Ott; Karl-Heinz	Lawrenceville	NJ		
Kwagh; Jae-Gyu	Fairless Hills	PA		
Stockton; Gerald W.	Yardley	PA		

US-CL-CURRENT: 435/232; 435/183, 435/189, 536/23.2

CLAIMS:

What is claimed is:

1. A variant plant acetohydroxy acid synthase (AHAS) protein comprising at least one mutation at an amino acid residue corresponding to an amino acid residue selected from the group consisting of M53, R128, I330, and any combination of the foregoing, of SEQ ID NO:1, where said variant plant AHAS protein is more resistant to an herbicide than a wild-type AHAS protein.
2. A variant AHAS protein as defined in claim 1, wherein said herbicide is selected from the group consisting of an imidazolinones, sulfonylureas, triazolopyrimidine, sulfomamides, pyrimidyl-oxy-benzoic acids, sulfamoylureas, sulfonylcarboxamides, and combinations thereof.
3. A variant AHAS protein as defined in claim 1, wherein said AHAS protein is derived from *Arabidopsis thaliana*.
4. A variant AHAS protein as defined in claim 1, wherein said substitution is selected from the group consisting of Met53Trp, Met53Glu, Met53Ile, Met53His, Arg128Ala, Arg128Glu, Ile330Phe, an identical substitution at an amino acid residue of another plant AHAS protein at an amino acid position aligned with any of the foregoing, or a combination of any of the foregoing.
5. A variant AHAS protein as defined in claim 1, wherein said variant AHAS protein has catalytic activity that is more resistant to at least one herbicide than is wild type AHAS.
6. A variant AHAS protein as defined in claim 1, wherein said variant AHAS has more than about 20% of the catalytic activity of wild-type AHAS.

7. A variant AHAS protein as defined in claim 1, wherein said variant AHAS is at least 2-fold more resistant to imidazolinone-based herbicides than to sulfonylurea-based herbicides.

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**Search Results** - Record(s) 71 through 78 of 78 returned.

☐ 71. Document ID: US 6107063 A

Using default format because multiple data bases are involved.

L1: Entry 71 of 78

File: USPT

Aug 22, 2000

US-PAT-NO: 6107063

DOCUMENT-IDENTIFIER: US 6107063 A

TITLE: Production of L-isoleucine by means of recombinant microorganisms with deregulated threonine dehydratase

DATE-ISSUED: August 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moeckel; Bettina	Duesseldorf			DE
Eggeling; Lothar	Juelich			DE
Sahm; Hermann	Juelich			DE

US-CL-CURRENT: 435/116; 435/252.32, 435/252.33, 435/320.1, 536/23.2, 536/23.7

Full	Title	Citation	Front	Review	Classification	Date	Reference	Classifications	Abstracts	Claims	KWIC	Draw. De
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☐ 72. Document ID: US 5962670 A

L1: Entry 72 of 78

File: USPT

Oct 5, 1999

US-PAT-NO: 5962670

DOCUMENT-IDENTIFIER: US 5962670 A

TITLE: Promoters for enhancing plant productivity

DATE-ISSUED: October 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Walling; Linda L.	Claremont	CA		
Pautot; Veronique	Gif sur Yvette			FR
Gu; Yong-Qiang	West Lafayette	IN		
Chao; Wun Shaw	Pullman	WA		

US-CL-CURRENT: 536/23.6; 536/24.1, 800/287

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KWIC	Draw. De
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☐ 73. Document ID: US 5928937 A

L1: Entry 73 of 78

File: USPT

Jul 27, 1999

US-PAT-NO: 5928937

DOCUMENT-IDENTIFIER: US 5928937 A

TITLE: Structure-based designed herbicide resistant products

DATE-ISSUED: July 27, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kakefuda; Genichi	Yardley	PA		
Ott; Karl-Heinz	Lawrenceville	NJ		
Kwagh; Jae-Gyu	Fairless Hills	PA		
Stockton; Gerald W.	Yardley	PA		

US-CL-CURRENT: 435/320.1; 435/419, 536/23.6, 536/24.1, 800/295, 800/298

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KWIC	Draw. De
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☐ 74. Document ID: US 5925594 A

L1: Entry 74 of 78

File: USPT

Jul 20, 1999

US-PAT-NO: 5925594

DOCUMENT-IDENTIFIER: US 5925594 A

TITLE: Method to overcome the antagonistic interactions of herbicides

DATE-ISSUED: July 20, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Whatley; Thomas	East Windsor	NJ		
Frazier; Todd L.	Aurora	CO		
Krueger; Roger W.	Baldwin	MO		

US-CL-CURRENT: 504/105; 504/130

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KWIC	Draw. De
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☐ 75. Document ID: US 5905186 A

L1: Entry 75 of 78

File: USPT

May 18, 1999

US-PAT-NO: 5905186

DOCUMENT-IDENTIFIER: US 5905186 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Chimeric plant genes based on upstream regulatory elements of helianthinin

DATE-ISSUED: May 18, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thomas; Terry	College Station	TX		
Freyssinet; Georges	Saint Cyr au Mont d'Or			FR
Lebrun; Michel	Lyons			FR
Bogue; Molly	Strasbourg			FR

US-CL-CURRENT: 800/281; 435/320.1, 435/412, 435/414, 435/415, 435/418, 435/419,  
435/428, 536/23.6, 536/24.1, 800/278, 800/287, 800/300, 800/300.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Annotations	Claims	KWIC	Drawings
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☐ 76. Document ID: US 5853973 A

L1: Entry 76 of 78

File: USPT

Dec 29, 1998

US-PAT-NO: 5853973

DOCUMENT-IDENTIFIER: US 5853973 A

TITLE: Structure based designed herbicide resistant products

DATE-ISSUED: December 29, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Takefuda; Genichi	Yardley	PA		
Ott; Karl-Heinz	Lawrenceville	NJ		
Kwagh; Jae-Gyu	Fairless Hills	PA		
Stockton; Gerald W.	Yardley	PA		

US-CL-CURRENT: 435/4; 435/232, 435/252.3, 435/252.33, 435/29, 435/320.1, 435/6,  
536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Annotations	Claims	KWIC	Drawings
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☐ 77. Document ID: US 5824865 A

L1: Entry 77 of 78

File: USPT

Oct 20, 1998

US-PAT-NO: 5824865

DOCUMENT-IDENTIFIER: US 5824865 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Chimeric plant genes based on upstream regulatory elements of helianthinin

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thomas; Terry	College Station	TX		
Freyssinet; Georges	Saint Cyr au Mont d'Or			FR
Lebrun; Michel	Lyon			FR
Bogue; Molly	Strasbourg			FR

US-CL-CURRENT: 800/281; 435/320.1, 536/23.6, 536/24.1, 800/287, 800/294, 800/300,  
800/300.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Abstract	References	Claims	KMC	Draw D
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☐ 78. Document ID: US 5290753 A

L1: Entry 78 of 78

File: USPT

Mar 1, 1994

US-PAT-NO: 5290753

DOCUMENT-IDENTIFIER: US 5290753 A

TITLE: Method for the prevention of crop injury in the presence of synergistic pesticide combinations

DATE-ISSUED: March 1, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Newhouse; Keith E.	Bensalem	PA		
Schaefer; Thomas J.	Levittown	PA		
Cary; Gail E.	Lawrenceville	NJ		

US-CL-CURRENT: 504/214; 504/215, 504/247, 504/253, 514/75, 800/266, 800/275,  
800/278

Full	Title	Citation	Front	Review	Classification	Date	Reference	Abstract	References	Claims	KMC	Draw D
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acetohydroxy acid synthase.clm.

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**Search Results - Record(s) 1 through 9 of 9 returned.**

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☐ 1. Document ID: US 20040033238 A1**Using default format because multiple data bases are involved.**

L9: Entry 1 of 9

File: PGPB

Feb 19, 2004

PGPUB-DOCUMENT-NUMBER: 20040033238

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040033238 A1

TITLE: Selectable genetic marker for use in pasteurellaceae species

PUBLICATION-DATE: February 19, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mulks, Martha H.	Williamston	MI	US	
Martin, Paul R.	Sun Lakes	AZ	US	

US-CL-CURRENT: 424/200.1; 435/252.3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 2. Document ID: US 20030186352 A1

L9: Entry 2 of 9

File: PGPB

Oct 2, 2003

PGPUB-DOCUMENT-NUMBER: 20030186352

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030186352 A1

TITLE: Apicomplexan chorismate synthase sequences and an inhibitor of the shikimate pathway

PUBLICATION-DATE: October 2, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
McLeod, Rima L.	Chicago	IL	US	
Kirisits, Michael	Chicago	IL	US	
Roberts, Craig W.	Kirklee	CO	GB	
Mack, Doug	Centennial	IL	US	
Mui, Ernest	Chicago	GA	US	



Barnwell, John	Stone Mountain	FL	US
Dame, John	Gainesville	MD	US
Carlton, Jane	Rockville	CA	US
Bartlett, Paul	Oakland	WA	US
Parle, Suzanna	Seattle		US

US-CL-CURRENT: 435/32; 435/258.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw. D
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☐ 3. Document ID: US 20030054436 A1

L9: Entry 3 of 9

File: PGPB

Mar 20, 2003

PGPUB-DOCUMENT-NUMBER: 20030054436

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054436 A1

TITLE: STAPHYLOCOCCUS AUREUS POLYNUCLEOTIDES AND SEQUENCES

PUBLICATION-DATE: March 20, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
KUNSCH, CHARLES A.	GAITHERSBURG	MD	US	
CHOI, GIL A.	ROCKVILLE	MD	US	
BARASH, STEVEN C.	ROCKVILLE	MD	US	
DILLON, PATRICK J.	GAITHERSBURG	MD	US	
FANNON, MICHAEL R.	SILVER SPRING	MD	US	
ROSEN, CRAIG A.	LAYTONSVILLE	MD	US	

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 536/23.1, 536/23.7

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw. D
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☐ 4. Document ID: US 20030023386 A1

L9: Entry 4 of 9

File: PGPB

Jan 30, 2003

PGPUB-DOCUMENT-NUMBER: 20030023386

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030023386 A1

TITLE: Metabolome profiling methods using chromatographic and spectroscopic data in pattern recognition analysis

PUBLICATION-DATE: January 30, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Aranibar, Nelly	Lawrenceville	NJ	US
Ott, Karl-Heinz	Lawrenceville	NJ	US
Stockton, Gerald	Yardley	PA	US

US-CL-CURRENT: 702/19

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 5. Document ID: US 20020197605 A1

L9: Entry 5 of 9

File: PGPB

Dec 26, 2002

PGPUB-DOCUMENT-NUMBER: 20020197605

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197605 A1

TITLE: Novel Polynucleotides

PUBLICATION-DATE: December 26, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nakagawa, Satoshi	Tokyo		JP	
Mizoguchi, Hiroshi	Tokyo		JP	
Ando, Seiko	Tokyo		JP	
Hayashi, Mikiro	Tokyo		JP	
Ochiai, Keiko	Tokyo		JP	
Yokoi, Haruhiko	Tokyo		JP	
Tateishi, Naoko	Tokyo		JP	
Senoh, Akihiro	Tokyo		JP	
Ikeda, Masato	Tokyo		JP	
Ozaki, Akio	Hofu-shi		JP	

US-CL-CURRENT: 435/6; 435/287.2, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 6. Document ID: US 6737237 B1

L9: Entry 6 of 9

File: USPT

May 18, 2004

US-PAT-NO: 6737237

DOCUMENT-IDENTIFIER: US 6737237 B1

TITLE: Antimicrobial agents, diagnostic reagents, and vaccines based on unique Apicomplexan parasite components

DATE-ISSUED: May 18, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McLeod; Rima L.	Chicago	IL		
Roberts; Craig W.	Glasgow			GB
Roberts; Fiona	Glasgow			GB
Johnson; Jennifer J.	Stillwater	MN		
Kirisits; Michael	Chicago	IL		
Ferguson; David	Tackley Oxford			GB
Lyons; Russell	Glasgow			GB
Mui; Ernest	Chicago	IL		
Mack; Doug	Riverside	IL		
Samuel; Benjamin	Chicago	IL		
Gornicki; Piotr	Chicago	IL		
Zuther; Ellen	Beuhy			DE

US-CL-CURRENT: 435/6; 435/19, 435/254.2, 435/320.1, 435/69.1, 435/7.2, 435/7.22,  
536/23.7, 536/23.74

Full	Title	Citation	Front	Review	Classification	Date	Reference	Abstracts	Attachments	Claims	KWIC	Draw. De
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☐ 7. Document ID: US 6699654 B1

L9: Entry 7 of 9

File: USPT

Mar 2, 2004

US-PAT-NO: 6699654

DOCUMENT-IDENTIFIER: US 6699654 B1

TITLE: Antimicrobial agents diagnostic reagents, and vaccines based on unique  
apicomplexan parasite components

DATE-ISSUED: March 2, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McLeod; Rima L. W.	Chicago	IL	60637	
Roberts; Craig W.	Kirklee, Glasgow, G12 OTW	Scotland		GB
Roberts; Fiona	Kirklee, Glasgow, G12 OTW	Scotland		GB
Johnson; Jennifer J.	Bolingbrook	IL	60440	
Mets; Laurens	Wilmette	IL	60091	

US-CL-CURRENT: 435/4; 435/6, 435/7.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Abstracts	Attachments	Claims	KWIC	Draw. De
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☐ 8. Document ID: US 6696561 B1

L9: Entry 8 of 9

File: USPT

Feb 24, 2004

US-PAT-NO: 6696561

DOCUMENT-IDENTIFIER: US 6696561 B1

TITLE: Corynebacterium glutamicum genes encoding proteins involved in membrane synthesis and membrane transport

DATE-ISSUED: February 24, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pompejus; Markus	Freinsheim			DE
Kroger; Burkhard	Limburgerhof			DE
Schroder; Hartwig	Nussloch			DE
Zelder; Oskar	Speyer			DE
Haberhauer; Gregor	Limburgerhof			DE

US-CL-CURRENT: 536/23.7; 530/350

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KMC	Draw D
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☐ 9. Document ID: US 6531316 B1

L9: Entry 9 of 9

File: USPT

Mar 11, 2003

US-PAT-NO: 6531316

DOCUMENT-IDENTIFIER: US 6531316 B1

TITLE: Encryption of traits using split gene sequences and engineered genetic elements

DATE-ISSUED: March 11, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Patten; Phillip A.	Menlo Park	CA		
Lassner; Michael	Davis	CA		

US-CL-CURRENT: 435/455; 435/440, 435/463, 435/6, 435/91.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KMC	Draw D
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synechocystis and acetohydroxy acid synthase

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## WEST Search History

DATE: Monday, August 02, 2004

<b>Hide?</b>	<b>Set Name</b>	<b>Query</b>	<b>Hit Count</b>
		<i>DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ</i>	
<input type="checkbox"/>	L9	synechocystis and acetohydroxy acid synthase	9
<input type="checkbox"/>	L8	synechocystis and aceto hydroxy acid synthase	0
<input type="checkbox"/>	L7	synechocystis with ilvG	0
<input type="checkbox"/>	L6	cyanobacterium with ilvG	0
<input type="checkbox"/>	L5	cyanobacterium ilvG	0
<input type="checkbox"/>	L4	acetohydroxy acid synthase with cyanobacterium	1
<input type="checkbox"/>	L3	acetohydroxy acid synthase with cyanobacterium.clm.	0
<input type="checkbox"/>	L2	L1 and cyanobacteri?	2
<input type="checkbox"/>	L1	acetohydroxy acid synthase.clm.	78

END OF SEARCH HISTORY

=> file medline hcaplus biosis biotechds scisearch embase		
COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	0.24	0.45

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=> s cyanobacterium and acetohydroxy acid synthase?  
 L1 26 CYANOBACTERIUM AND ACETOHYDROXY ACID SYNTHASE?

=> dup rem l1  
 PROCESSING COMPLETED FOR L1  
 L2 10 DUP REM L1 (16 DUPLICATES REMOVED)

=> d l2 1-10 ibib ab

L2 ANSWER 1 OF 10 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
 ACCESSION NUMBER: 2002-06244 BIOTECHDS

TITLE: New cyanobacterial nucleic acid fragments encoding  
 acetohydroxyacid synthase (AHAS) or phytoene desaturase  
 (PDS), useful for conferring herbicide, fungicide or  
 insecticide resistance, and for identifying AHAS or PDS  
 inhibitors;  
 Synechocystis sp. enzyme conferring plant pesticide  
 resistance

AUTHOR: KAKEFUDA G; KOOP H; STURNER S; ZHEN R  
 PATENT ASSIGNEE: AMERICAN CYANAMID CO  
 PATENT INFO: WO 2002000915 3 Jan 2002  
 APPLICATION INFO: WO 2000-US20338 27 Jun 2000  
 PRIORITY INFO: US 2000-214705 27 Jun 2000  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 OTHER SOURCE: WPI: 2002-139930 [18]

AB DERWENT ABSTRACT:

NOVELTY - New isolated and purified polynucleotides comprising a sequence  
 containing 1909 base pairs (bp) (I), 566 bp (II) or 1735 bp (III) where  
 all sequences are fully defined in the specification.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for  
 target site gene identification in cyanobacteria, the successful  
 development of various protocols for High-Through-Put molecular  
 manipulation of Synechocystis comprising: (1) lead compound  
 identification; (2) generation and selection of resistant mutant; (3)  
 isolation of genomic DNA from resistant cell lines; (4) primer design and  
 polymerase chain reaction (PCR) amplification of gene fragments from  
 Synechocystis; and (5) high throughput genetic transformation and target  
 site gene identification.

BIOTECHNOLOGY - Preferred Polynucleotide: The polynucleotide  
 comprising (I) or (II) is a cyanobacterial nucleic acid fragments

encoding a herbicide resistant acetohydroxyacid synthase (AHAS) larger subunit gene. The isolated and purified polynucleotide comprising (III) is a cyanobacterial nucleic acid fragments encoding a herbicide resistant mutant phytoene desaturase (PDS) gene. The cyanobacteria is *Synechocystis* PCC 6803.

ACTIVITY - Plant growth protectant.

MECHANISM OF ACTION - None given in the source material.

USE - The isolated and purified polynucleotides are useful for controlling plant traits via nuclear or plastome transformation, e.g. for conferring herbicide, fungicide or insecticide resistance. In particular, these are useful in identifying novel PDS and AHAS inhibitors, and in plant transformations for conferring resistance and cross-resistance to certain bleaching herbicides and AHAS-inhibiting herbicides.

EXAMPLE - Genomic DNA was prepared from 6 *Synechocystis* EMS resistant cell lines. A 1.7 kilo base pair (kb) Genomic DNA fragment encompassing the phytoene desaturase (PDS) was amplified using Genomic DNA as a template. Polymerase chain reaction (PCR) amplified PDS gene fragments were subsequently subcloned into the Invitrogen TOPO TA Cloning vector pCR2.1-TOPO to obtain plasmid pCR2.1-TOPO-PDS. Three independent clones were picked and sequenced using the dRhodoamine Terminator Cycle Sequencing Kit. The complete sequence of the novel mutant form PDS gene was identified as having 1735 base pairs (bp). A probe for identifying the *Synechocystis* acetohydroxyacid synthase (AHAS) gene was generated by PCR with degenerate primers. A genomic library from *Synechocystis* PCC 6803 in the Lambda ZAP vector was screened for the AHAS gene. The phagemid DNA obtained for the library screening process was labeled pSyn23/1. pSyn23/1 was double digested with the restriction enzymes EcoRI and Cla I to produce a 3 kb fragment. The isolated fragment was ligated into pBluescript II and transformed into DH5 alpha, giving pSyn23/1-I. This AHAS clone was sequenced using fmol DNA Sequencing System and a set of eight gene-specific primers plus the T3 sequencing primer located in the pBluescript II vector. The resulting sequence of a large subunit of AHAS comprised 1909 bp. A *Synechocystis* AHAS small subunit nucleic acid fragment was also cloned from a genomic DNA library of **cyanobacterium** *Synechocystis* PCC 6803. The resultant *Synechocystis* sp. Strain PCC 6803 revealed a sequence comprising 566 bp. (70 pages)

L2 ANSWER 2 OF 10 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 2002327246 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12070685  
TITLE: Biosynthesis of the branched-chain amino acids in the **cyanobacterium** *Synechocystis* PCC6803: existence of compensatory pathways.  
AUTHOR: Kouhen Odile Maestri-El; Joset Francoise  
CORPORATE SOURCE: Laboratoire de Chimie Bacterienne-CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France.  
SOURCE: Current microbiology, (2002 Aug) 45 (2) 94-8.  
Journal code: 7808448. ISSN: 0343-8651.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200302  
ENTRY DATE: Entered STN: 20020619  
Last Updated on STN: 20030225  
Entered Medline: 20030224

AB Complementation of an *E. coli* mutant auxotrophic for the branched-chain amino acids (BCAA)--valine, leucine, and isoleucine--by the *ilvG* gene (slr2088) of the **cyanobacterium** *Synechocystis* PCC6803 indicates that this gene encodes an active alpha-acetohydroxy acid synthase. Differences of response of the recombinants to the addition of the essential amino acids suggested a lower specificity for the initial reaction of the valine/leucine chain than for the isoleucine one. Inactivation of *ilvG* in *Synechocystis* led to a leaky phenotype,



suggesting a capacity to compensate the auxotrophies by other processes. This observation is discussed in view of the general difficulty of obtaining auxotrophs in cyanobacteria.

L2 ANSWER 3 OF 10 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 2000279950 EMBASE  
TITLE: Regulation by external pH and stationary growth phase of the acetolactate synthase from *Synechocystis* PCC6803.  
AUTHOR: Maestri O.; Joset F.  
CORPORATE SOURCE: F. Joset, Laboratoire de Chimie Bacterienne, CNRS, 31 Chemin J. Aiguier, 13402 Marseilles Cedex 20, France. joset@ibsm.cnrs-mrs.fr  
SOURCE: Molecular Microbiology, (2000) 37/4 (828-838).  
Refs: 51  
ISSN: 0950-382X CODEN: MOMIEE  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Several characteristics identify the protein encoded by the *alsS* gene [sll1981 in Cyanobase (<http://www.kazusa.or.jp/cyano/cyano.html>)] of *Synechocystis* PCC6803 as an acetolactate synthase. The AlsS protein is about 60% homologous to the AlsS from *Bacillus subtilis* or other bacteria. These enzymes condense two pyruvates to form acetolactate, implicated in pH homeostasis via the acetoin-2,3-butanediol pathway or in valine biosynthesis. Transcriptional fusions revealed that *alsS* was induced at the onset of stationary phase, as in *B. subtilis*, a situation leading to an increase in the pH(out) to above 11 in *Synechocystis*. This is the first cyanobacterial gene showing a dependence on pH for its expression. Induction was also obtained by the presence of > 100 mM Na<sup>+</sup>, the effect being prevented by amiloride, in agreement with Na<sup>+</sup>/H<sup>+</sup> exchange in the pH homeostasis process. Homology of the *Synechocystis* AlsS protein to the close family of **acetohydroxy acid synthases** (including one in *Synechocystis*) is around 30%. These enzymes are involved in the parallel routes for valine/leucine and isoleucine biosynthesis. No phenotype of auxotrophy for any of these amino acids was associated with a null mutation in the *Synechocystis alsS* gene. The AlsS enzyme did not complement the isoleucine deficiency of an **acetohydroxy acid synthase**-deficient *Escherichia coli* mutant.

L2 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 1997:768387 HCAPLUS  
DOCUMENT NUMBER: 128:21915  
TITLE: Sustained production of amino acids by immobilized analog-resistant mutants of a **cyanobacterium** *Anacystis nidulans* BD-1  
AUTHOR(S): Bagchi, Suvendra Nath; Rao, Nandula Seshgiri  
CORPORATE SOURCE: Department of Biological Sciences, Rani Durgavati University, Jabalpur, 482 001, India  
SOURCE: Journal of Microbiology and Biotechnology (1997), 7(5), 341-344  
CODEN: JOMBES; ISSN: 1017-7825  
PUBLISHER: Korean Society for Applied Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Batch cultures of *Anacystis nidulans* BD-1 resistant to azaleucine and fluorotyrosine produced and liberated a wide range of amino acids, notably glutamic acid, alanine, phenylalanine, leucine, isoleucine, cysteine and methionine. Sustained liberation for prolonged periods was achieved after immobilization on calcium alginate and the net concn. in the medium was 0.18.apprx.0.2 g l<sup>-1</sup>. While **acetohydroxy acid synthase** in azaleucine-resistant mutant lost leucine- and isoleucine-sensitivity, fluorotyrosine-resistant strain turned

phenylalanine activating. The activities of nitrate assimilating enzymes were also higher in the mutants and were relaxed from ammonium-repression. The metabolic adjustments involved in amino acid overprod. are discussed.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 1996:111190 HCAPLUS

DOCUMENT NUMBER: 124:170225

TITLE: De-regulated assimilation and over-production of amino acids in analog-resistant mutants of a **cyanobacterium**, *Phormidium uncinatum*

AUTHOR(S): Rao, N.S.; Shakila, T.M.; Bagchi, S.N.

CORPORATE SOURCE: Department of Biological Sciences, R.D. University, Jabalpur, 482 001, India

SOURCE: World Journal of Microbiology & Biotechnology (1995), 11(6), 665-8  
CODEN: WJMBEY; ISSN: 0959-3993

PUBLISHER: Rapid Science Publishers

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutant strains of *Phormidium uncinatum* resistant to fluorophenylalanine, azatryptophan, fluorotyrosine and azaleucine accumulated a wide range of amino acids, notably glutamic acid, lysine, tyrosine and phenylalanine, and exhibited de-regulated valine and phenylalanine transport. While **acetohydroxy acid synthase** in azaleucine-resistant mutants lost valine- and leucine-sensitivity, 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) synthase and prephenate dehydratase in arom. analog-resistant strains became phenylalanine-insensitive and shikimate and prephenate dehydrogenases were activated by tyrosine. In addn., activities of nitrate-assimilating enzymes were higher in the mutants, which also exhibited increased nitrogen, protein and phycocyanin contents. The proteins in the mutants were better digested upon enzymic-treatments and feeding trials than those of the wild type, indicating that they are usable as single-cell protein.

L2 ANSWER 6 OF 10 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 92381487 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1512571

TITLE: Molecular characterization of the genes encoding **acetohydroxy acid synthase** in the **cyanobacterium** *Spirulina platensis*.

AUTHOR: Milano A; De Rossi E; Zanaria E; Barbierato L; Ciferri O; Riccardi G

CORPORATE SOURCE: Dipartimento di Genetica e Microbiologia A. Buzzati Traverso, Pavia, Italy.

SOURCE: Journal of general microbiology, (1992 Jul) 138 ( Pt 7) 1399-408.  
Journal code: 0375371. ISSN: 0022-1287.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-M75906; GENBANK-M75907

ENTRY MONTH: 199209

ENTRY DATE: Entered STN: 19921018

Last Updated on STN: 19921018

Entered Medline: 19920930

AB The enzyme **acetohydroxy acid synthase** (AHS), which catalyses the first common step in the biosynthesis of isoleucine, leucine and valine, has been demonstrated to be present in *Spirulina platensis* in two isoenzymic forms. The complete nucleotide sequences of the genes *ilvX* and *ilvW* encoding these two enzymes have been determined. Sequence analysis revealed the presence of two open reading frames, of 1836 and 1737 nucleotides for *ilvX* and *ilvW*, respectively. The predicted

amino acid sequences of the two isoenzymes, compared with the Synechococcus PCC 7942 AHS enzyme and the large subunits of the Escherichia coli AHS I, II, III isoenzymes, revealed a notable degree of similarity. A small subunit has not been identified for either of the S. platensis AHS isoenzymes. Analysis by Northern blot hybridization demonstrated that the ilvX and ilvW genes are transcribed to give mRNA species of approximately 2.15 kb and 1.95 kb, respectively.

L2 ANSWER 7 OF 10 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
DUPLICATE 5

ACCESSION NUMBER: 1991-08162 BIOTECHDS

TITLE: Molecular cloning and expression of Spirulina platensis  
**acetoxyhydroxy-acid-synthase** genes  
in Escherichia coli;  
single cell protein-producing S. platensis isoleucine,  
leucine and valine biosynthesis gene cloning  
AUTHOR: Riccardi G; De Rossi E; Milano A; Forlani G; De Felice M  
LOCATION: Dipartimento di Genetica e Microbiologia 'A. Buzzati  
Traverso', via S. Epifanio 14, I-27100 Pavia, Italy.  
SOURCE: Arch.Microbiol.; (1991) 155 4, 360-65  
CODEN: AMICCW

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Spirulina platensis is potentially useful for single cell protein production. The coding sequence (ilvX) for S. platensis **acetoxyhydroxy-acid-synthase** (AHAS, EC-4.1.3.18) was isolated as a 4.2 kb ClaI fragment from a S. platensis Sau3AI gene bank in phage lambda-EMBL3 in Escherichia coli HB101. This DNA fragment was able to complement a suitable mutant of E. coli, PS1283 (lacking AHAS activity due to a Mu-1 insertion in the ilvB gene, a frameshift in ilvG and a deletion which includes ilvH). The gene was expressed when inserted into the ClaI site of plasmid pAT153 in either orientation to form plasmid pSpM1 and plasmid pSpM2, demonstrating that transcription of ilvX originated within the cloned fragment. The probe used for hybridization experiments was the corresponding gene from Anabaena sp. PCC7120. The same probe facilitated identification of a second putative gene encoding AHAS in the S. platensis gene bank. The ability to express a cyanobacterial enzyme in E. coli offers the opportunity to make use of the fast growth of the host for analysis of gene expression. (30 ref)

L2 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 6

ACCESSION NUMBER: 1991:225313 HCAPLUS

DOCUMENT NUMBER: 114:225313

TITLE: Biochemical evidence for multiple forms of  
**acetoxyhydroxy acid synthase**  
in Spirulina platensis

AUTHOR(S): Forlani, Giuseppe; Riccardi, Giovanna; De Rossi, Edda;  
De Felice, Maurilio

CORPORATE SOURCE: Dep. Genet. Microbiol. "A. Buzzati Traverso", Univ.  
Pavia, Pavia, I-27100, Italy

SOURCE: Archives of Microbiology (1991), 155(3), 298-302  
CODEN: AMICCW; ISSN: 0302-8933

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Two isoforms of **acetoxyhydroxy acid synthase** (AHAS), the first enzyme of the branched-chain amino acid biosynthetic pathway, were detected in cell-free exts. of the **cyanobacterium** S. platensis and were sep'd. by both ion-exchange chromatog. and hydrophobic interaction. Several biochem. properties of the 2 putative isoenzymes were analyzed. They differ for pH optimum, FAD requirement for both activity and stability, and for heat lability. The results were partially confirmed with the characterization of the enzyme extd. from a recombinant Escherichia coli strain transformed with 1 subcloned S. platensis AHAS gene. The approx. mol. mass of both AHAS activities, estd.

by gel filtration, indicates that they are distinct isoenzymes and not different oligomeric species or aggregates of identical subunits.

L2 ANSWER 9 OF 10 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN  
ACCESSION NUMBER: 91:145225 SCISEARCH  
THE GENUINE ARTICLE: FB010  
TITLE: BIOCHEMICAL-EVIDENCE FOR MULTIPLE FORMS OF  
**ACETOHYDROXY ACID SYNTHASE** IN  
SPIRULINA-PLATENSIS  
AUTHOR: FORLANI G (Reprint); RICCARDI G; DEROSI E; DEFELICE M  
CORPORATE SOURCE: UNIV PAVIA, DEPT GENET & MICROBIOL A BUZZATI TRAVERSO, VIA  
S EPIFANIO 14, I-27100 PAVIA, ITALY (Reprint); INT INST  
GENET & BIOPHYS, I-80125 NAPLES, ITALY  
COUNTRY OF AUTHOR: ITALY  
SOURCE: ARCHIVES OF MICROBIOLOGY, (1991) Vol. 155, No. 3, pp.  
298-302.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 19

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Two isoforms of **acetohydroxy acid synthase**  
(AHAS), the first enzyme of the branched-chain amino acids biosynthetic  
pathway, were detected in cell-free extracts of the **cyanobacterium**  
*Spirulina platensis* and separated both by ion-exchange chromatography and  
by hydrophobic interaction. Several biochemical properties of the two  
putative isozymes were analysed and it was found that they differ for pH  
optimum, FAD requirement for both activity and stability, and for heat  
lability. The results were partially confirmed with the characterization  
of the enzyme extracted from a recombinant *Escherichia coli* strain  
transformed with one subcloned *S. platensis* AHAS gene. The approximate  
molecular mass of both AHAS activities, estimated by gel filtration,  
indicates that they are distinct isozymes and not different oligomeric  
species or aggregates of identical subunits.

L2 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1988:109317 HCAPLUS  
DOCUMENT NUMBER: 108:109317  
TITLE: Detection and characterization of **acetohydroxy**  
**acid synthase** in *Spirulina platensis*  
AUTHOR(S): Riccardi, Giovanna; De Rossi, Edda; Nielsen, Erik; De  
Felice, Maurilio  
CORPORATE SOURCE: Dip. Genet. Microbiol. 'A. Buzzati Traverso', Pavia,  
I-27100, Italy  
SOURCE: FEMS Microbiology Letters (1988), 49(1), 13-17  
CODEN: FMLED7; ISSN: 0378-1097  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Growth of the **cyanobacterium** *S. platensis*, like that of many  
prokaryotic and eukaryotic organisms, is inhibited by low concns. of  
valine, one of the three end-products of the branched-chain amino acid  
biosynthetic pathway. The activity of **acetohydroxy acid**  
**synthase** (AHAS), the first common enzyme of the branched pathways  
in cell-free exts. from axenic *S. platensis* cultures, was assayed and  
partially characterized. Assays performed at various pH values showed two  
peaks of activity, both inhibited by valine. FAD was not required for  
enzyme activity but protected it during dialysis. Also investigated was  
whether the three amino acids were able to cause repression of AHAS  
synthesis; a significant drop in the enzyme-specific activity could be  
seen only when cultures were grown in the presence of valine. Chromatog.  
on hydroxylapatite showed one single peak of activity.

=> s ilvG and acetohydroxy acid synthase?

L3 67 ILVG AND ACETOHYDROXY ACID SYNTHASE?

```
=> dup rem l3
PROCESSING COMPLETED FOR L3
L4          30 DUP REM L3 (37 DUPLICATES REMOVED)

=> s l4 and synechococcus
L5          0 L4 AND SYNECHOCOCCUS

=> s synechocystis and acetohydroxy acid synthase?
L6          13 SYNECHOCYSTIS AND ACETOHYDROXY ACID SYNTHASE?

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7          4 DUP REM L6 (9 DUPLICATES REMOVED)

=> d l7 1-4 ibib ab
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L7 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
 ACCESSION NUMBER: 2002-06244 BIOTECHDS  
 TITLE: New cyanobacterial nucleic acid fragments encoding  
 acetohydroxyacid synthase (AHAS) or phytoene desaturase  
 (PDS), useful for conferring herbicide, fungicide or  
 insecticide resistance, and for identifying AHAS or PDS  
 inhibitors;

**Synechocystis** sp. enzyme conferring plant  
 pesticide resistance

AUTHOR: KAKEFUDA G; KOOP H; STURNER S; ZHEN R  
 PATENT ASSIGNEE: AMERICAN CYANAMID CO  
 PATENT INFO: WO 2002000915 3 Jan 2002  
 APPLICATION INFO: WO 2000-US20338 27 Jun 2000  
 PRIORITY INFO: US 2000-214705 27 Jun 2000  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 OTHER SOURCE: WPI: 2002-139930 [18]

AB DERWENT ABSTRACT:

NOVELTY - New isolated and purified polynucleotides comprising a sequence containing 1909 base pairs (bp) (I), 566 bp (II) or 1735 bp (III) where all sequences are fully defined in the specification.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for target site gene identification in cyanobacteria, the successful development of various protocols for High-Through-Put molecular manipulation of **Synechocystis** comprising: (1) lead compound identification; (2) generation and selection of resistant mutant; (3) isolation of genomic DNA from resistant cell lines; (4) primer design and polymerase chain reaction (PCR) amplification of gene fragments from **Synechocystis**; and (5) high throughput genetic transformation and target site gene identification.

BIOTECHNOLOGY - Preferred Polynucleotide: The polynucleotide comprising (I) or (II) is a cyanobacterial nucleic acid fragments encoding a herbicide resistant acetohydroxyacid synthase (AHAS) larger subunit gene. The isolated and purified polynucleotide comprising (III) is a cyanobacterial nucleic acid fragments encoding a herbicide resistant mutant phytoene desaturase (PDS) gene. The cyanobacteria is **Synechocystis** PCC 6803.

ACTIVITY - Plant growth protectant.

MECHANISM OF ACTION - None given in the source material.

USE - The isolated and purified polynucleotides are useful for controlling plant traits via nuclear or plastome transformation, e.g. for conferring herbicide, fungicide or insecticide resistance. In particular, these are useful in identifying novel PDS and AHAS inhibitors, and in plant transformations for conferring resistance and cross-resistance to certain bleaching herbicides and AHAS-inhibiting herbicides.

EXAMPLE - Genomic DNA was prepared from 6 **Synechocystis** EMS resistant cell lines. A 1.7 kilo base pair (kb) Genomic DNA fragment encompassing the phytoene desaturase (PDS) was amplified using Genomic

DNA as a template. Polymerase chain reaction (PCR) amplified PDS gene fragments were subsequently subcloned into the Invitrogen TOPO TA Cloning vector pCR2.1-TOPO to obtain plasmid pCR2.1-TOPO-PDS. Three independent clones were picked and sequenced using the dRhodoamine Terminator Cycle Sequencing Kit. The complete sequence of the novel mutant form PDS gene was identified as having 1735 base pairs (bp). A probe for identifying the **Synechocystis** acetohydroxyacid synthase (AHAS) gene was generated by PCR with degenerate primers. A genomic library from **Synechocystis** PCC 6803 in the Lambda ZAP vector was screened for the AHAS gene. The phagemid DNA obtained for the library screening process was labeled pSyn23/1. pSyn23/1 was double digested with the restriction enzymes EcoRI and Cla I to produce a 3 kb fragment. The isolated fragment was ligated into pBluescript II and transformed into DH5 alpha, giving pSyn23/1-I. This AHAS clone was sequenced using fmol DNA Sequencing System and a set of eight gene-specific primers plus the T3 sequencing primer located in the pBluescript II vector. The resulting sequence of a large subunit of AHAS comprised 1909 bp. A **Synechocystis** AHAS small subunit nucleic acid fragment was also cloned from a genomic DNA library of cyanobacterium **Synechocystis** PCC 6803. The resultant **Synechocystis** sp. Strain PCC 6803 revealed a sequence comprising 566 bp. (70 pages)

L7 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2002327246 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12070685  
 TITLE: Biosynthesis of the branched-chain amino acids in the cyanobacterium **Synechocystis** PCC6803: existence of compensatory pathways.  
 AUTHOR: Kouhen Odile Maestri-El; Joset Françoise  
 CORPORATE SOURCE: Laboratoire de Chimie Bactérienne-CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France.  
 SOURCE: Current microbiology, (2002 Aug) 45 (2) 94-8.  
 Journal code: 7808448. ISSN: 0343-8651.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200302  
 ENTRY DATE: Entered STN: 20020619  
 Last Updated on STN: 20030225  
 Entered Medline: 20030224

AB Complementation of an E. coli mutant auxotrophic for the branched-chain amino acids (BCAA)--valine, leucine, and isoleucine--by the *ilvG* gene (slr2088) of the cyanobacterium **Synechocystis** PCC6803 indicates that this gene encodes an active alpha-acetohydroxy acid synthase. Differences of response of the recombinants to the addition of the essential amino acids suggested a lower specificity for the initial reaction of the valine/leucine chain than for the isoleucine one. Inactivation of *ilvG* in **Synechocystis** led to a leaky phenotype, suggesting a capacity to compensate the auxotrophies by other processes. This observation is discussed in view of the general difficulty of obtaining auxotrophs in cyanobacteria.

L7 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN  
 ACCESSION NUMBER: 2001:217060 SCISEARCH  
 THE GENUINE ARTICLE: 407HM  
 TITLE: Protein trans-splicing to produce herbicide-resistant acetolactate synthase  
 AUTHOR: Sun L; Ghosh I; Paulus H; Xu M Q (Reprint)  
 CORPORATE SOURCE: New England Biolabs Inc, 32 Tozer Rd, Beverly, MA 01915 USA (Reprint); New England Biolabs Inc, Beverly, MA 01915 USA; Boston Biomed Res Inst, Watertown, MA 02472 USA  
 COUNTRY OF AUTHOR: USA  
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (MAR 2001) Vol. 67, No. 3, pp. 1025-1029.

Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW,  
WASHINGTON, DC 20036-2904 USA.  
ISSN: 0099-2240.

DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 17

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Protein splicing in trans has been demonstrated both in vivo and in vitro by biochemical and immunological analyses, but in vivo production of a functional protein by trans-splicing has not been reported previously. In this study, we used the DnaE intein from *Synechocystis* sp. strain PCC6803, which presumably reconstitutes functional DnaE protein by trans-splicing in vivo, to produce functional herbicide-resistant acetolactate synthase II (ALSII) from two unlinked gene fragments in *Escherichia coli*. The gene for herbicide-resistant ALSII was fused in frame to DnaE intein segments capable of promoting protein splicing in trans and was expressed from two compatible plasmids as two unlinked fragments. Cotransformation of *E. coli* with the two plasmids led to production of a functional enzyme that conferred herbicide resistance to the host *E. coli* cells. These results demonstrate the feasibility of expressing functional genes from two unlinked DNA loci and provide a model for the design of nontransferable transgenes in plants.

L7 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 2000497211 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10972805  
TITLE: Regulation by external pH and stationary growth phase of the acetolactate synthase from *Synechocystis* PCC6803.  
AUTHOR: Maestri O; Joset F  
CORPORATE SOURCE: Laboratoire de Chimie Bacterienne, CNRS, Marseilles, France.  
SOURCE: Molecular microbiology, (2000 Aug) 37 (4) 828-38.  
Journal code: 8712028. ISSN: 0950-382X.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200010  
ENTRY DATE: Entered STN: 20001027  
Last Updated on STN: 20001027  
Entered Medline: 20001019

AB Several characteristics identify the protein encoded by the *alsS* gene [sl11981 in Cyanobase (<http://www.kazusa.or.jp/cyano/cyano.html>)] of *Synechocystis* PCC6803 as an acetolactate synthase. The *AlsS* protein is about 60% homologous to the *AlsS* from *Bacillus subtilis* or other bacteria. These enzymes condense two pyruvates to form acetolactate, implicated in pH homeostasis via the acetoin-2, 3-butanediol pathway or in valine biosynthesis. Transcriptional fusions revealed that *alsS* was induced at the onset of stationary phase, as in *B. subtilis*, a situation leading to an increase in the pH<sub>out</sub> to above 11 in *Synechocystis*. This is the first cyanobacterial gene showing a dependence on pH for its expression. Induction was also obtained by the presence of > 100 mM Na<sup>+</sup>, the effect being prevented by amiloride, in agreement with Na<sup>+</sup>/H<sup>+</sup> exchange in the pH homeostasis process. Homology of the *Synechocystis* *AlsS* protein to the close family of **acetohydroxy acid synthases** (including one in *Synechocystis*) is around 30%. These enzymes are involved in the parallel routes for valine/leucine and isoleucine biosynthesis. No phenotype of auxotrophy for any of these amino acids was associated with a null mutation in the *Synechocystis* *alsS* gene. The *AlsS* enzyme did not complement the isoleucine deficiency of an **acetohydroxy acid synthase**-deficient *Escherichia coli* mutant.

=> d his

(FILE 'HOME' ENTERED AT 14:37:25 ON 02 AUG 2004)

FILE 'STNGUIDE' ENTERED AT 14:37:50 ON 02 AUG 2004

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT  
14:40:20 ON 02 AUG 2004

L1	26 S CYANOBACTERIUM AND ACETOHYDROXY ACID SYNTHASE?
L2	10 DUP REM L1 (16 DUPLICATES REMOVED)
L3	67 S ILVG AND ACETOHYDROXY ACID SYNTHASE?
L4	30 DUP REM L3 (37 DUPLICATES REMOVED)
L5	0 S L4 AND SYNECHOCOCCUS
L6	13 S SYNECHOCYSTIS AND ACETOHYDROXY ACID SYNTHASE?
L7	4 DUP REM L6 (9 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
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FULL ESTIMATED COST	49.45	49.90
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL
	ENTRY	SESSION
CA SUBSCRIBER PRICE	-2.94	-2.94

STN INTERNATIONAL LOGOFF AT 14:48:30 ON 02 AUG 2004